

***CANCER ASSESSMENT DOCUMENT***

EVALUATION OF THE CARCINOGENIC POTENTIAL OF  
***LINDANE***  
**PC. Code: 009001**

Final Report

November 29, 2001

**CANCER ASSESSMENT REVIEW COMMITTEE**  
**HEALTH EFFECTS DIVISION**  
**OFFICE OF PESTICIDE PROGRAMS**

---

DATA PRESENTATION:

\_\_\_\_\_  
Suhair Shallal, Toxicologist

DOCUMENT PREPARATION:

\_\_\_\_\_  
Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE:

(Signature indicates concurrence with the  
assessment unless otherwise stated).

Karl Baetcke

\_\_\_\_\_

William Burnam

\_\_\_\_\_

Marion Copley

\_\_\_\_\_

Kerry Dearfield

\_\_\_\_\_

Vicki Dellarco

\_\_\_\_\_

Virginia Dobozy

\_\_\_\_\_

Richard Hill

\_\_\_\_\_

Yiannakis Ioannou

\_\_\_\_\_

Tim McMahon

\_\_\_\_\_

Nancy McCarroll

\_\_\_\_\_

Esther Rinde

\_\_\_\_\_

Jess Rowland

\_\_\_\_\_

Joycelyn Stewart

\_\_\_\_\_

Clark Swentzel

\_\_\_\_\_

Linda Taylor

\_\_\_\_\_

Yin-Tak-Woo

\_\_\_\_\_

NON-COMMITTEE MEMBERS IN ATTENDANCE

(Signature indicates concurrence with the  
pathology report and statistical analysis of data,  
respectively)

John M. Pletcher, Pathology Consultant

\_\_\_\_\_

Lori Brunsman, Statistical Analysis

\_\_\_\_\_

## CONTENTS

Executive Summary.....	iii
I. Introduction.....	1
II. Background Information.....	1
III. Evaluation of Carcinogenicity.....	2
1. Combined Chronic Toxicity & Carcinogenicity Study in CD-1 Mice.....	2
2. Carcinogenicity Study in Agouti, Pseudo Agouti and Black Mice.....	7
3. NTP Carcinogenicity Study in B6C3F1 Mice.....	8
4. Carcinogenicity Study in Wistar Rats.....	11
5. NTP Chronic Toxicity & Carcinogenicity Study in Osborne-Mendel Rats.....	13
IV. Toxicology.....	14
1. Metabolism.....	14
2. Mutagenicity.....	15
3. Structure Activity Relationship.....	16
4. Subchronic and chronic Toxicity.....	16
5. Mode of Action Studies.....	18
V. Committee's Assessment of the Weight-of-the Evidence.....	19
VI. Classification of Carcinogenic Potential.....	22
VII. Quantification of Carcinogenic Potential.....	22
V. Bibliography.....	23

## EXECUTIVE SUMMARY

Lindane (gamma isomer of hexachlorocyclohexane, -HCH) has been previously classified by the Cancer Assessment Group of the Office of Research and Development (CAG/ORD, 1985) as a group “B2/C” carcinogen based on an increased incidence of mouse liver tumors. In 1993, the RfD/Peer Review Committee (1993) determined that the mouse carcinogenicity data were inadequate because of major deficiencies associated with the available studies. The Toxicology Endpoint Selection (TES) Committee concluded that a new carcinogenicity study in mice was needed to make a determination of the carcinogenic potential of lindane (TES, 1994).

On May 30, 2001, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs met to evaluate the carcinogenic potential of lindane. At this meeting, the CARC could not make a determination of the carcinogenic potential of lindane because the NTP studies were limited in value and it was uncertain if the study on Agouti, Pseudoagouti and Black mice with limited data could be used for regulatory purposes. In addition, the CARC was informed that new histopathology data would be submitted. The Committee also requested additional information including results of a 90-day subchronic range-finding study in CD-1 mice, an earlier RfD Committee report and analyses of the older studies on lindane.

At the September 13, 2001 meeting, the Committee reevaluated all the available information/data including the old and the newly gathered information that was previously not available for review. [A list of CARC members who attended one or both meetings on lindane is presented on page # i. These meetings were held jointly by teleconference with Pesticide Management Regulatory Agency (PMRA), Health Canada, Canada]. The chronic toxicity/carcinogenicity studies were conducted using 5 different strains of mice and two strains of rats. The dietary doses administered in these studies were as follows:

CD-1 mice (50/sex/dose): 0, 10, 40, or 160 ppm for 78 weeks (0, 1.3, 5.2, and 21 mg/kg/day for males and 0, 1.8, 7.1, and 26.8 mg/kg/day for females, respectively).

Female Agouti, Pseudoagouti and Black mice (36-96 animals per strain): 0 or 160 ppm for 24 months

B6C3F1 mice (50 /sex/dose): 0, 80 or 160 ppm for 80 weeks

Wistar rats (50/sex/dose): 0, 1, 10, 100, or 400 ppm for 2 years (0, 0.05, 0.47, 4.81, and 19.66 mg/kg/day for males and 0, 0.06, 0.59, 6.00, and 24.34 mg/kg/day for females, respectively).

Osborne-Mendel rats (50/sex/dose): For males: 320 or 640 ppm for 38 weeks and 160 or 320 ppm for the remaining 42 weeks. For females: 320 or 640 ppm for 2 weeks and 160 or 320 ppm for 49 weeks then for the remaining 29 weeks the dose was lowered to 80 or 160 ppm. Matched controls consisted of 10/sex.

**The CARC concluded that lindane was carcinogenic only to female CD-1 mice based on the following:**

- CD-1 female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 160 ppm (26.8 mg/kg/day) dose group with the controls, for lung alveolar-bronchiolar adenomas and combined adenomas/carcinomas, all at  $p < 0.05$ . The incidence of lung adenomas was slightly outside the historical control range. However, the increased incidence of carcinomas was not dose-dependent and the tumor response was variable. Lindane was not carcinogenic to male mice. The dosing at the highest level was adequate and not excessive based on increased incidences of centrilobular hepatocellular hypertrophy and eosinophilic foci of cellular alteration in males and a slight increase in bronchiolar-alveolar adenomas in females.
- At 160 ppm, both the treated female Agouti and Pseudoagouti mice had an increase in benign lung adenomas; the treated Agouti mice also had an increased incidence of liver adenomas. No statistical analyses of tumor data were conducted. There was no increase in the incidence or decrease in latency period for liver tumors in Black and Pseudoagouti strains of mice. There was evidence of increased liver weights and an increased incidence of Clara cell hyperplasia in Agouti and Black strains of mice. However, the study was conducted on few animals, only a single dose and sex were tested, no statistical analyses of tumor data were presented and the results of the study were not adequately reported. The Committee concluded that although the liver effects appear to suggest that a dose of 160 ppm was adequate, additional dose groups could have provided confirmatory information.
- The CARC could not assess the carcinogenicity of lindane in B6C3F<sub>1</sub> male and female mice because the data reporting was inadequate and there were no indications of toxicity in high dose females. Moreover, the use of only 10 mice per sex for the control group compromised the usefulness of the study.

**The Committee concluded that the increased incidence of lung tumors in female mice of three strains was treatment-related** because the statistically significant increase in lung adenomas in female CD-1 mice was corroborated with the increase in lung tumors in two genetically susceptible strains of mice. Although there is some evidence of liver tumor induction in these genetically susceptible strains of mice, no evidence of liver tumors was noted in CD-1 mice. Nevertheless, the evidence of hepatotoxicity (increased liver weight, hypertrophy and increased incidence of liver foci in both sexes) and promoting activity, indicates that the liver, in addition to lung is a major target organ of toxicity.

- The CARC determined that lindane was not carcinogenic to male and female Wistar rats and the results of the study in Osborne Mendel rats were difficult to interpret and were not useful in determining the carcinogenic potential of lindane in that strain of rat.
- The results of a battery of acceptable mutagenicity assays indicate that lindane has a low concern for mutagenicity. These studies satisfy pre-1991 FIFRA guideline requirements. The

Committee recommended that the dominant lethal assay be repeated to determine if there is a genetic component to the reproductive (germ cell) effects reported for lindane.

- The technical HCH and the alpha-isomer are classified as category “B2” (probable human carcinogen). The beta-isomer is classified as a group “C” (possible human carcinogen) while the delta and epsilon isomers are classified as group “D” (not classifiable as to human carcinogenicity) .
- No mechanistic studies were submitted to support the mode of action for lung tumor induction in mice.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified lindane into the category “**Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential**” because lindane caused an increased incidence of benign lung tumors in female mice only. The Committee further recommended that quantification of human cancer risk is not required.

## I. INTRODUCTION

Lindane (gamma isomer of hexachlorocyclohexane,  $\gamma$ -HCH) was previously classified by the Cancer Assessment Group of the ORD (1985) as a group "B2/C" carcinogen based on an increased incidence of mouse liver tumors; the calculated unit risk ( $Q1^*$ ) was  $1.1 \text{ (mg/kg/day)}^{-1}$  human equivalents. The other isomers of hexachlorocyclohexane are classified in IRIS. Technical HCH and the alpha-isomer are classified as "B2", probable human carcinogens. The beta-isomer is classified as "C", possible human carcinogen. The delta and epsilon isomers are classified as D, not classifiable as to human carcinogenicity.

In 1993, the RfD/Peer Review Committee determined that the mouse carcinogenicity data were insufficient because of major deficiencies associated with all available studies. The TES committee concluded that a new carcinogenicity study in mice was needed to make a determination of the carcinogenic potential of lindane (TES, 1994).

On May 30, 2001, the HED Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs met to evaluate the carcinogenic potential of lindane. At this meeting, the CARC could not make a determination of the carcinogenic potential of lindane because the NTP studies were limited in value and the published study on Agouti, Pseudoagouti and Black mice could not be used for regulatory purposes. The Committee, therefore, requested additional information including results of a 90-day subchronic range-finding study in CD-1 mice, an earlier RfD Committee report and analyses of the older studies on lindane. At the September 13, 2001 meeting, the Committee met to reevaluate the carcinogenic potential of lindane based on the available old and new information/data. At this meeting, information/data were presented by Dr. Suhair Shallal of Reregistration Branch 4. These data included a new mouse carcinogenicity study in CD-1 mice submitted by the registrant, a published study in Agouti, Pseudoagouti and Black mice, NCI studies in B6C3F1 mice and Osborne-Mendel rats and a 2-year chronic/carcinogenicity study in Wistar rats. In addition, carcinogenicity and genetic toxicology data on structurally-related compounds were presented.

## II. BACKGROUND INFORMATION

Lindane (PC. Code is 009001 and CAS Number is 58-89-9) is a broad-spectrum organochlorine compound used on a wide range of soil-dwelling and plant-eating (phytophagous) insects. Its chemical structure is provided below.

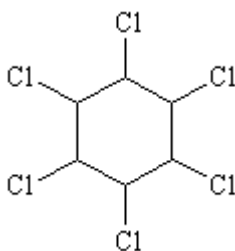


Figure 1. Chemical Structure of  $\gamma$ -HCH

The technical HCH consists of  $\gamma$ -isomer: 65-67%  $\delta$ -isomer: 11-13%,  $\epsilon$ -isomer: 13-15%  $\theta$ -isomer: 3-5%,  $\alpha$ -isomer: 4-6% and other isomers: <1%. Lindane is a  $\gamma$ -isomer (1, 2, 3 hexachlorocyclohexane). The other isomers are:  $\delta$ -isomer (1, 2, 3 hexachlorocyclohexane);  $\epsilon$ -isomer (1, 2, 3 hexachlorocyclohexane);  $\theta$ -isomer (1, 2, 3 hexachlorocyclohexane) and  $\alpha$ -isomer: (1, 2, 3 hexachlorocyclohexane).

Worldwide, lindane is commonly used on a wide variety of crops, in warehouses, in public health to control insect-borne diseases, and (with fungicides) as a seed treatment. Lindane is also presently used in lotions, creams, and shampoos for the control of lice and mites (scabies) in humans; these pharmaceutical uses are regulated by FDA. **In the U.S., the only registered food/feed use is seed treatment for field and vegetable crops.**

Lindane may be found in formulations with a host of fungicides and insecticides. Labels for products containing the chemical must bear the Signal Word WARNING. Some formulations of lindane are classified as Restricted Use Pesticides (RUP), and as such may only be purchased and used by certified pesticide applicators. **Lindane is no longer manufactured in the U.S., and most agricultural and dairy uses have been canceled because of concerns about its potential carcinogenicity.**

### III. EVALUATION OF CARCINOGENICITY STUDIES

#### 1. Combined Chronic Toxicity/Carcinogenicity Study with Lindane in CD-1 Mice

Reference: Lindane, carcinogenicity study by dietary administration to CD-1 mice for 78 weeks (2000), final report (vols. 1-4). Huntingdon Life Sciences Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England, Report no. 00 3512, Huntingdon Life Sciences Project identity no. CIL/021, **MRID 45291402**.

#### Experimental Design

Lindane (99.78% a.i., batch no. HLS 96/1) was administered to groups of 50 Crl: CD-1<sup>®</sup> (ICR)BR mice/sex/dose in the diet at concentrations of 0, 10, 40, or 160 ppm. The test diets were given for 78 weeks. The concentrations of 10, 40, or 160 ppm resulted in mean daily compound intakes for males of 1.3, 5.2, and 21 mg/kg/day and for females of 1.8, 7.1, and 27 mg/kg/day, respectively.

#### Discussion of Tumor Data

##### Tumor Analyses

As shown in Table 1, there was no statistically significant increase in tumors in male mice (Brunsman, 2001). The incidence of liver tumors was not statistically significant in either male or female CD-1 mice (Table 1).



**Table 1.** CD-1 Mice- Male and Female Liver Tumor Rates<sup>+</sup> and Exact Trend Test and Fisher's Exact Test Results (p values)- (Brunsmann 2001)

<i>ppm</i>	<i>0</i>	<i>10</i>	<i>40</i>	<i>160</i>	<i>0</i>	<i>10</i>	<i>40</i>	<i>160</i>
<i>mg/kg/day</i>	<i>0</i>	<i>1.3</i>	<i>5.2</i>	<i>20.5</i>	<i>0</i>	<i>1.8</i>	<i>7.1</i>	<i>26.8</i>
	<i>Males</i>				<i>females</i>			
<i>Tumor Type</i>	<i>Liver Tumor</i>							
<i>Adenoma</i>	<i>10<sup>a</sup>/48</i>	<i>10/46</i>	<i>9/43</i>	<i>13/46</i>	<i>0/47</i>	<i>0/45</i>	<i>0/47</i>	<i>1<sup>a</sup>/48</i>
<i>%</i>	<i>(21)</i>	<i>(22)</i>	<i>(21)</i>	<i>(28)</i>	<i>(0)</i>	<i>(0)</i>	<i>(0)</i>	<i>(2)</i>
<i>p =</i>	<i>0.1730</i>	<i>0.5570</i>	<i>0.5962</i>	<i>0.2752</i>	<i>0.2567</i>	<i>1.000</i>	<i>1.000</i>	<i>0.5053</i>
<i>Carcinoma</i>	<i>4/48</i>	<i>1/46</i>	<i>1/43</i>	<i>2<sup>b</sup>/46</i>	<i>No carcinomas were observed in females</i>			
<i>%</i>	<i>(8)</i>	<i>(2)</i>	<i>(2)</i>	<i>(4)</i>				
<i>p =</i>	<i>0.4046</i>	<i>0.1943</i>	<i>0.2167</i>	<i>0.3592</i>				
<i>Combined</i>	<i>13/48</i>	<i>11/46</i>	<i>10/43</i>	<i>15/46</i>				
<i>%</i>	<i>(27)</i>	<i>(24)</i>	<i>(23)</i>	<i>(33)</i>				
<i>p =</i>	<i>0.1817</i>	<i>0.4543</i>	<i>0.4304</i>	<i>0.3594</i>				

<sup>+</sup>Number of tumorbearing animals/Number of animals examined, excluding those that died before week 53.

<sup>a</sup>First adenoma observed in males at week 67, dose 0 ppm; First adenoma observed in females at week 80, dose 160 ppm

<sup>b</sup>First carcinoma observed in males at week 53, dose 10 ppm; no carcinomas were observed in females.

Note: Significance of trend denoted at control.  
Significance of pair-wise comparison with control denoted at dose level.

If \*, then  $p < 0.05$ . If \*\*, then  $p < 0.01$ .

Treatment for up to 78 weeks with lindane resulted in a statistically significant increase in the incidence of bronchiolar-alveolar adenomas and an increased incidence of carcinomas in female Crl:CD-1 mice; however, the increased incidence of carcinomas was not dose-dependent and tumor response was variable. Female mice had significant increasing trends and significant differences in the pair-wise comparisons of the 160 ppm dose group with the controls for lung alveolar-bronchiolar adenomas and combined adenomas/carcinomas, all at  $p < 0.05$  (Table 2a). The incidence of pulmonary adenomas in the control group was at the low end of the range in historical controls (6%) and the incidence in females administered the high dose (23%) was slightly outside of the high end of the range for the historical controls (6%-19%) (MRID 45291402).

**Table 2a.** Male and Female Lung Alveolar-Bronchiolar Tumor Rates<sup>+</sup> and Exact Trend Test and Fisher's Exact Test Results (p values)- Initial Diagnosis (Brunsman, 2001)

<i>ppm</i>	<i>0</i>	<i>10</i>	<i>40</i>	<i>160</i>	<i>0</i>	<i>10</i>	<i>40</i>	<i>160</i>
<i>mg/kg/day</i>	<i>0</i>	<i>1.3</i>	<i>5.2</i>	<i>20.5</i>	<i>0</i>	<i>1.8</i>	<i>7.1</i>	<i>26.8</i>
	<i>males</i>				<i>females</i>			
<i>Tumor Type</i>	<i>Lung Alveolar-Bronchiolar Tumors</i>							
<i>Adenoma</i>	<i>16<sup>a</sup>/49</i>	<i>15/48</i>	<i>11/49</i>	<i>8/48</i>	<i>3<sup>a</sup>/48</i>	<i>7/46</i>	<i>7/47</i>	<i>11/48</i>
<i>%</i>	<i>(33)</i>	<i>(31)</i>	<i>(22)</i>	<i>(17)</i>	<i>(6)</i>	<i>(15)</i>	<i>(15)</i>	<i>(23)</i>
<i>p =</i>	<i>0.0270*<sup>n</sup></i>	<i>0.5278</i>	<i>0.1830</i>	<i>0.0554</i>	<i>0.0274*</i>	<i>0.1412</i>	<i>0.1497</i>	<i>0.0200*</i>
<i>Carcinoma</i>	<i>0/49</i>	<i>1/48</i>	<i>3<sup>b</sup>/49</i>	<i>0/48</i>	<i>1/48</i>	<i>2<sup>b</sup>/46</i>	<i>2/47</i>	<i>1/48</i>
<i>%</i>	<i>(0)</i>	<i>(2)</i>	<i>(6)</i>	<i>(0)</i>	<i>(2)</i>	<i>(4)</i>	<i>(4)</i>	<i>(2)</i>
<i>p =</i>	<i>0.3138</i>	<i>0.4948</i>	<i>0.1211</i>	<i>1.0000</i>	<i>0.4361</i>	<i>0.4839</i>	<i>0.4920</i>	<i>0.7526</i>
<i>Combined</i>	<i>16/49</i>	<i>16/48</i>	<i>14/49</i>	<i>8/48</i>	<i>4/48</i>	<i>8/46</i>	<i>9/47</i>	<i>12/48</i>
<i>%</i>	<i>(33)</i>	<i>(33)</i>	<i>(29)</i>	<i>(17)</i>	<i>(8)</i>	<i>(17)</i>	<i>(19)</i>	<i>(25)</i>
<i>p =</i>	<i>0.0186*<sup>n</sup></i>	<i>0.5574</i>	<i>0.4134</i>	<i>0.0554</i>	<i>0.0389*</i>	<i>0.1573</i>	<i>0.1080</i>	<i>0.0264*</i>

<sup>a</sup> first adenoma observed in males at week 33, dose 0 ppm and in females at week 44, dose 0 ppm

<sup>b</sup> first carcinoma observed males at week 65, dose 40 ppm and in females at week 53, dose 10 ppm

<sup>n</sup> Negative trend

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level

If \*, then  $p < 0.05$ . If \*\*, then  $p < 0.01$ .

A new report on the results of resectioning of lungs of female mice was later submitted by the Registrant (MRID 45470601). The results showed the presence of two additional pulmonary adenomas in the controls and two in the high dose group. The number of pulmonary adenomas and combined adenomas/carcinomas in Group 4 remained still statistically significant (Table 2b), and was biologically significant, being well above the historical control range for this strain of mouse. The incidence in the controls and in the two intermediate dose groups was within the historical control range (6%-19%) for pulmonary adenomas in CD-1 females. **It is, however, difficult to compare old tumor data versus the combined analyses based on the old and new findings without knowing the exact procedure involved in resectioning the lungs and why resectioning of the lung tissue was necessary. Therefore, without judging the validity of the new sectioning versus the original report, the end results appear to be the same.**

Table 2b. Lindane - CD-1 Mouse Study  
Female Lung Alveolar-Bronchiolar Tumor Rates<sup>†</sup> [Additional Histopathology PLUS  
 Original Diagnoses] and Exact Trend Test and Fisher's Exact Test Results (p  
 values)-Results of Re-sectioning (Brunsman, 2001)

	<u>Dose (ppm)</u>			
	0	10	40	160
Adenomas (%)	5 <sup>a</sup> /48 (10)	7/46 (15)	7/47 (15)	13/48 (27)
p =	0.0165*	0.3492	0.3644	0.0326*
Carcinomas (%)	1/48 (2)	2 <sup>b</sup> /46 (4)	2/47 (4)	1/48 (2)
p =	0.4361	0.4839	0.4920	0.7526
Combined (%)	6/48 (12)	8/46 (17)	9/47 (19)	14/48 (29)
p =	0.0235*	0.3535	0.2723	0.0384*

<sup>†</sup>Number of tumor bearing animals/Number of animals examined, excluding those that died before week 44.

<sup>a</sup>First adenoma observed at week 44, dose 0 ppm.

<sup>b</sup>First carcinoma observed at week 53, dose 10 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If \*, then  $p < 0.05$ . If \*\*, then  $p < 0.01$ .

### Non-Neoplastic Lesions

At 78 weeks, there were increases in the incidences of centrilobular hepatocyte hypertrophy (control, 6%; 160 ppm, 30%;  $p < 0.01$ ) and eosinophilic focus/foci of hepatocellular alteration (control, 4%; 160 ppm, 16%;  $p < 0.05$ ) in high-dose males compared to the control group (Table 3). No microscopic liver changes were seen in females

**Table 3. Non-Neoplastic Lesions in CD-1 Mice Fed Lindane**

<i>Dose (ppm)</i>	<i>0</i>	<i>10</i>	<i>40</i>	<i>160</i>	<i>0</i>	<i>10</i>	<i>40</i>	<i>160</i>
<i>mg/kg/day</i>	<i>0</i>	<i>1.3</i>	<i>5.2</i>	<i>20.5</i>	<i>0</i>	<i>1.8</i>	<i>7.1</i>	<i>26.8</i>
<i>males</i>					<i>females</i>			
centrilobular hepatocyte hypertrophy	3/50	2/50	7/50	15/50	0/50	0/50	0/50	0/50
eosinophilic focus/foci of hepatocyte. alterations	2/50	1/50	5/50	8/50	0/50	0/50	0/50	0/50
lung epithelial hyperplasia	1/50	1/50	1/50	2/50	1/50	0/50	0/50	2/50
lung congestion	16/50	12/50	16/50	11/50	6/50	13/50	8/50	13/50
Thyroid- dilated follicles	9/49	4/16	3/16	14/50	2/50	1/19	0/17	3/50
LN Bronchial- increased cellularity	1/16	0/17	1/15	0/11	0/12	1/15	2/15	3/14

#### Adequacy of the Dosing for Assessment of Carcinogenicity

There was no significant change in body weight and the survival analyses indicated no statistically significant incremental changes with increasing doses of lindane in male or female mice (Brunsman, 2001). All dose groups and controls had 68% survival at study termination.

Histopathology revealed an increased incidence of liver lesions in male mice. The LOAEL was 160 ppm for males (20.5 mg/kg/day) and females (26.8 mg/kg/day) based on liver hypertrophy in males and a slight increase in bronchiolar-alveolar adenomas in females.

The doses selected for the above chronic/carcinogenicity study were based on the results of a range-finding subchronic toxicity study (MRID 45424301). In this study, lindane (99.78% a.i.) was administered to 10 CD-1 mice/sex/dose at dietary levels of 0, 40, 80, 160, 320 ppm (0, 5.7, 12.2, 22.8 and 46.2 and 0, 8.9, 16.0, 32.9, and 62.6 mg/kg/day in males and females, respectively). Body weight gain was reduced by 27% in males and 9% in females at the highest dose. The four females in the highest dose (320 ppm) group that died during treatment period had hepatocellular hypertrophy and karyomegaly in the liver and Clara cell hypertrophy as well as congestion in the lungs. These findings were also seen in treated animals in the 160 and 320 ppm dose groups that were sacrificed at study termination; therefore, these deaths were considered to be treatment-related. The early deaths in the 320 ppm dose group indicate that this dose was excessive. Based on the results of this study the majority of the CARC concluded that the dose levels of 0, 10, 40 160 ppm selected for the two-year carcinogenicity study in mice appeared to be adequate. However, a few members felt that the animals could have tolerated a higher dose, based on the results of chronic study.

## 2. Combined Chronic Toxicity/Carcinogenicity Study with Lindane in Agouti, Pseudoagouti and Black Mice

*Reference:* Wolff, G.L. et al. Tumorigenic responses to lindane in mice: potentiation by a dominant mutation, NCTR, Jefferson, AR ; Carcinogenesis 8(12): 1889-97 (1987).

### Experimental Design

In an NCI study, three strains of female mice, Agouti, Pseudoagouti, and Black, were administered lindane at dietary concentrations of 0 or 160 ppm. Groups of 36-96 animals per strain were continuously fed treated or control diets for up to 24 months. Additional groups of 48-96 Agouti and Black mice were fed treated or control diets for 6 months and then fed control diet for 6 or 18 months (recovery).

---

### Tumor Analysis:

No evidence for an increased incidence or a decreased latency of liver tumors was observed for the black strain at any time during the 24 months of study or for the Pseudoagouti strain through the 18 month sacrifice. At 18 months, 0/34 control and 12/36 (33%) of the treated Agouti mice developed hepatocellular adenomas; one carcinoma each in the treated and control groups was noted. Both the treated Agouti and Pseudoagouti strains had clear increases in adenomas and slight increases in carcinomas at 24 months. The incidence rates for the control and treated Agouti groups were 9% and 35%, respectively, for adenomas and 13% and 17%, respectively, for carcinomas. The incidence rates for the control and treated Pseudoagouti groups were 5% and 12%, respectively, for adenomas and 2% and 5%, respectively, for carcinomas.

Increases in Clara cell hyperplasia were noted in the lung at all sacrifice intervals for each strain and the incidence of lung tumors was increased in later months for the Agouti and Pseudoagouti strains. The percentage of mice with Clara cell hyperplasia in the control and treated groups was 6-31% and 72-92%, respectively, for the Agouti; 6-17% and 50-79%, respectively, for the Pseudoagouti; and 0-14% and 56-90%, respectively, for the Black strain. Lung tumors for the Agouti strain occurred in 0% of the control and 17% of the treated animals at 18 months and 4% of the control and 19% of the treated animals at 24 months. Lung tumors in the Pseudoagouti strain occurred in 6% of the controls and 14% of the treated animals at 24 months. After recovery, the incidences of Clara cell hyperplasia (Agouti and Black mice) and lung tumors (Agouti mice) remained slightly elevated as compared with the controls.

### Non-neoplastic lesions:

No clinical signs of toxicity and no survival information were reported. No apparent effects on body weights or food consumption were observed, but only limited data were presented. When compared with untreated controls at 6 and 12 months, benzo(a)pyrene monooxygenase

activity in the liver was increased 1.61-1.84x in the Agouti, 2.71-2.78x in the Pseudoagouti, and 2.07-2.09x in the Black strains. Liver weights were increased 14.7-31.2% in the Agouti, 13.5-22.0% in the Pseudoagouti, and 12.2-16.4% in the Black strains at sacrifice intervals up to 24 months. Following the recovery period, liver weights of the treated mice were similar to the controls.

#### Adequacy of the dose:

Only two dose groups were tested in this study, 0 and 160 ppm. The CARC concluded that although the liver effects appear to suggest that a dose of 160 ppm was adequate, additional dose groups may have provided confirmatory information. A more thorough reporting of the clinical signs would have been useful in definitive determination of adequacy of dose.

### 3. NTP Combined Chronic Toxicity/Carcinogenicity Study with Lindane in Mice

Reference: NCI, Carcinogenesis Program, Bethesda, MD; DHEW Pub # (NIH) 77-814, 1977.

#### Experimental Design:

Groups of 50 B6C3F<sub>1</sub> mice/sex were administered lindane at dietary concentrations of 80 or 160 ppm for 80 weeks then observed for an additional 10-11 weeks. Matched controls consisted of 10 mice/sex. For statistical analysis, 40 untreated mice/sex were pooled from four other bioassays of other test chemicals.

#### Discussion of tumor data:

The incidence of hepatocellular carcinoma in low-dose males (19/49) was increased significantly ( $p=0.001$ ) when compared with pooled controls (5/49). The incidence of hepatocellular carcinoma in high-dose male mice (9/46) was not significantly different than the matched (2/10) or pooled controls.

#### Non-neoplastic lesions

The non-neoplastic lesions are presented in Table 4 below. There were only slight increases in liver inflammation in males and spleen hyperplasia in females. Higher incidences of microscopic changes in the uterus and ovaries of treated mice were noted; however, no clear dose response was found.

Table 4. Non-neoplastic lesions in B6C3F<sub>1</sub> male and female mice

Dose	0	80	160	0	80	160
	males			females		
liver, inflammation/ swelling	0/10	0/49	5/46	0/10	0/47	0/46
spleen, hyperplasia	0/10	1/50	0/47	0/8	2/49	4/48
uterus, hyperplasia	N/A			0/7	3/44	4/43
ovary , inflamation				3/7	14/42	10/46

#### Adequacy of Dosing for Assessment of Carcinogenicity

Body weight was unaffected by the test material. No food or water consumption data was provided. The CARC concluded that the use of pooled controls compromised the usefulness of the study and the available data were inadequate to make an assessment of the carcinogenic potential of lindane.

Table 5 provides a comparison of other available mouse studies and their deficiencies.

Table 5. Mouse Carcinogenicity Studies, their Results and Deficiencies<sup>1</sup>

Study design/deficiencies/classification	Results																									
<p>1. Carcinogenicity - CF-1 mouse Walker and Thorpe as published in Food Cosmetic Toxicology 11:433-442,1973.</p> <p>Supplementary. Data in summary tables were not supported by individual animal data. Test material cannot be validated. Only one dose level which produced severe toxicity was used. Study was run concurrently with dieldrin, DDT, phenobarbitone</p>	<p><b>Considered positive for liver tumors.</b></p> <table><tr><td></td><td colspan="2">Males</td><td colspan="2">Females</td></tr><tr><td></td><td>Control(45)</td><td>BHC(29)</td><td>Control(44)</td><td>BHC(29)</td></tr><tr><td>Adenoma</td><td>20%</td><td>38%</td><td>23%</td><td>34%</td></tr><tr><td>Carcinoma</td><td>4%</td><td>55%</td><td>0</td><td>34%</td></tr><tr><td>Total</td><td>24%</td><td>93%**</td><td>23%</td><td>69%**</td></tr></table> <p>[data are % of animals with tumor, the number in-( ) is the number of mice per sex examined.]</p> <p>** P &lt; 0.01 study author's statistics.</p> <p>CFI strain mouse, Dose levels tested: 0, 400 ppm and beta-BHC for 105 to 109 weeks.</p>		Males		Females			Control(45)	BHC(29)	Control(44)	BHC(29)	Adenoma	20%	38%	23%	34%	Carcinoma	4%	55%	0	34%	Total	24%	93%**	23%	69%**
	Males		Females																							
	Control(45)	BHC(29)	Control(44)	BHC(29)																						
Adenoma	20%	38%	23%	34%																						
Carcinoma	4%	55%	0	34%																						
Total	24%	93%**	23%	69%**																						
<p>2. Carcinogenicit-dd mouse Hamada, Yutani and Miya as published in GANN 64:511-3(1973).</p> <p>Supplementary. Data were available in summary form only. Very small number (only 3 or 4) of animals were dosed per group. The survival was poor and dosing period was only 32 weeks. Test material cannot be validated. Study was run concurrently with alpha, beta and gamma isomers of BHC.</p>	<p><b>considered positive</b></p> <p>Three of four males and one of three females receiving pure gamma isomer at 600 ppm were said to develop "hepatoma" or liver tumors. None of the controls or mice dosed with 100 or 300 ppm developed these tumors.</p> <p>dd strain mice, dose levels 0, 100, 300 or 600 ppm of gamma, alpha or beta hexachlorocyclohexane or crude "BHC" for 36 to 38 weeks.</p>																									

<p>3. Oncogenicity - B6C3F1 mouse NCI, No.: NCI-CG-TR-14, 1977</p> <p>Supplementary: Use of only 10 mice per sex for the control group compromised the usefulness of the study. Data were in summary tables only. There were no indications of toxicity at high dose. Test material cannot be validated.</p>	<p><b>Considered positive at low dose only.</b> Hepatocellular Carcinomas</p> <table><tr><th>Dose Level</th><th colspan="2">Males</th><th colspan="2">Females</th></tr><tr><td>Control</td><td>10</td><td>2(20%)</td><td>10</td><td>0</td></tr><tr><td>80 ppm</td><td>49</td><td>19(39%)</td><td>47</td><td>2(4%)</td></tr><tr><td>160 ppm</td><td>46</td><td>9(20%)</td><td>46</td><td>4(7%)</td></tr></table> <p>NCI study conclusion is that the chemical is not positive for liver tumors. Conclusion corroborated by Vesselinovich and Carlborg. The CAG of ORD considers low dose group positive. Dose levels tested were 0, 80 and 160 ppm for 80 weeks with 10 weeks recovery. B6C3CF<sub>1</sub> strain mice.</p>	Dose Level	Males		Females		Control	10	2(20%)	10	0	80 ppm	49	19(39%)	47	2(4%)	160 ppm	46	9(20%)	46	4(7%)
Dose Level	Males		Females																		
Control	10	2(20%)	10	0																	
80 ppm	49	19(39%)	47	2(4%)																	
160 ppm	46	9(20%)	46	4(7%)																	
<p>4. Oncogenicity -Chbb-NMRI mouse Boehringer Sohn Ingelheim am Rhein, No Study No.: February 25. 1975</p> <p>Supplementary: Insufficient raw data were used to support conclusions. There was no verification of identity of test material and no evidence of toxicity at high dose level.</p>	<p>No evidence of liver neoplasms.</p> <p>Dose levels tested 0, 12.5, 25 and 50 ppm.</p> <p>Chbb-NMRI strain mice.</p>																				
<p>5. Oncogenicity - mice (strain unspecified) Ito , Nagasaki, Arie, Sughara and Makiura, Nara Medical University - No Study No.: as published in J. National Cancer Institute 51:817- 826, 1972 . Supplementary/Invalid: No individual animal data were provided, test was conducted for only 24 weeks. There was no verification of the test material.</p>	<p>gamma isomer was not shown to increase liver tumors. Alpha isomer was positive.</p> <p>Dose levels tested 0, 100, 250 or 500 ppm for 24 weeks.</p> <p>Strain was not specified.</p>																				
<p>6. oncogenicity - ICR-JCL mouse Goto, Hattori, Miyagawa and Enomoto, Gakushin University, as published in Chemosphere 1(6):279-282 1972. No Study No., Supplementary: No individual animal data were available and there was no verification of the test material. Single dose level for only 26 weeks. Other isomers were tested at the same time. No information on survival or reactions to treatment was available. Test material was not validated as lindane.</p>	<p><b>considered positive</b> Liver tumors developed in 5 of 10 mice dosed with gamma isomer after 26 weeks.</p> <p>Dose level tested: 0 and 600 ppm, other isomers of HCH also tested.</p> <p>ICR-JCL strain mouse.</p>																				
<p>7. Carcinogenicit - dd mouse Nagasaki, Tonrii, Mega, Marugami and Ito, Nara Medical University, as published in Topics in Chemical Carcinogenesis, 1972 No Study No.: Supplementary: Technical ECCE (mixture of isomers) was used, not lindane. Data were available in summary tables only. Only males were tested.</p>	<p>"Hepatoma" resulted in response to 660 ppm of the test material (mixture of isomers).</p> <p>Dose levels tested: 0, 6.6, 66 and 660 ppm.</p> <p>dd strain of mice, only males tested.</p>																				
<p>8. Carcinogenicity - mouse Wolff and colleagues. AS published in Carcinogenesis 8(12):1889-1892 , 1987 Supplementary: Data are in summary tables only. No verification of the test material. Only females tested. Only a single dose tested. No data on clinical observations, body weight or survival. This strain may metabolize lindane at a slower rate with resulting accumulation in tissue.</p>	<p><b>Considered positive in two of three strains</b> Liver and lung adenomas and liver carcinomas in "pseudoagouti" and "yellow" but not in black normal mice.</p> <p>Dose levels tested 0 and 160 ppm.</p> <p>Strains as indicated above.</p>																				

<sup>1</sup> This table developed by John Doherty (1993 RfD document) has been slightly modified.



## 5. Carcinogenicity Study in Rats

### Reference:

Aymes, S.J. 1993. Lindane: Combined carcinogenicity and toxicity study by dietary administration to Wistar rats for 104 weeks. Addendum to final report (Adrenal histopathology - additional investigations). Life Sciences Research, England. Study No. 90/CIL002/0839. June 2, 1993. MRID 42891201. Unpublished.

Aymes, S.J. 1989. Combined carcinogenicity and toxicity study by dietary administration to Wistar rats for 104 weeks. Life Sciences Research, England. Study No. 90/CIL002/0839. November 7, 1989. MRID 41853701. Unpublished.

Aymes, S.J. 1989. Lindane: Combined carcinogenicity and toxicity study by dietary administration to Wistar rats for 104 weeks - Interim report week 0-26. Life Sciences Research, England. Study No. 88/CIL002/816. March 7, 1989. MRID 41094101. Unpublished.

### Experimental Design

Lindane (99.75% a.i., Lot no. DA433) was administered in the diet to groups of 50 male and 50 female Wistar rats at concentrations of 0, 1, 10, 100, or 400 ppm for 2 years. Corresponding delivered doses were 0, 0.05, 0.47, 4.81, and 19.66 mg/kg/day, respectively, for males and 0, 0.06, 0.59, 6.00, and 24.34 mg/kg/day, respectively, for females. An additional 15 rats/sex/group were designated for interim sacrifices at 30 days and 26 weeks.

### Discussion of Tumor Data

Male rats were identified as having adrenal pheochromocytomas (Table 6). The percentages of animals with adrenal tumors in the 0, 1, 10, 100, and 400 ppm groups were 14, 16, 16, 6, and 24% for benign tumors, respectively, and 0, 0, 6, 8, and 2% for malignant tumors, respectively. Statistical significance was not reached by relevant tests.

When compared to historical controls, the incidence of adrenal pheochromocytomas in the current study slightly exceeded that of the historical control at the HDT (400 ppm). The range of adrenal pheochromocytomas observed in the historical control data was 4/50 to 11/50 (8% - 22%) for male rats examined in four studies conducted in 1990. Of the 18 studies in the historical control data, 6 were performed in 1990; the other 12 were performed between 1986 and 1988.

### Non-Neoplastic Lesions

The incidence rate of periacinar hepatocytic hypertrophy was significantly ( $p < 0.01$ ) increased

in the 100 and 400 ppm groups with 25/50 males and 19/50 females at 100 ppm and in 40/50 males and 43/50 females at 400 ppm compared with the vehicle control. No treatment-related histopathological lesions were observed in the spleen or bone marrow.

Kidney lesions in males indicative of alpha 2 globulin accumulation were observed in animals treated with 10 ppm; but since this effect is species (rat) specific, it was not considered relevant to human health risk assessment.

**Table 6. Percentage (%) of animals with adrenal pheochromocytomas**

<b>Control (ppm)</b>	<b>Benign</b>	<b>Malignant</b>	<b>Both (B &amp; M)</b>
0	14	0	14
1	16	0	16
10	16	6	18
100	6	8	14
400	24	2	26
<b>Adjusted Rates<sup>1</sup></b>			
0	29	0	29
1	39	0	39
10	33.5	15.2	38.6
100	12.1	21.4	31.0
400	52.9	2.9	54.2

Historical controls: Benign- 8 to 22% and Malignant- 0 to 2%

<sup>1</sup> Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for inter-current mortality.

#### Adequacy of Dosing for Assessment of Carcinogenicity

CARC concluded that the doses tested were considered to be adequate and not excessive in both sexes. This was based on decreased survival, decreased body weight gains, decreased food consumption, and increased spleen and liver weights correlated with periportal hepatocyte hypertrophy in both sexes at the high-dose, relative to the controls.

Final body weights of the high-dose males were significantly (-14%; p = 0.05) less than the

controls. Body weights and body weight gains for the treated females were similar to the controls throughout the study. Total food consumption for the entire study was similar to the control levels.

Platelet counts were significantly increased in males at 100 and 400 ppm at week 12 and in males and females at week 24, but not at later time points. High-dose males and females had significant decreases in red blood cell parameters at week 104 as compared with the controls.

Significant changes in clinical chemistry parameters were observed in high-dose males and females during the first year of the study. Inorganic phosphorous and calcium were increased in males and females; the cholesterol and urea were increased in females; and the albumin/globulin ratio was decreased in females. All parameters were similar to the control levels by week 104.

High-dose males and females had increased absolute and relative liver weights at all interim sacrifices, although statistical significance was not always reached. At study termination, absolute and relative liver weights were significantly increased in high-dose males and females. At 100 ppm, absolute and relative liver weights were increased for both sexes.

#### 6. NTP Combined Chronic Toxicity/Carcinogenicity Study with Lindane in Rats

Reference: NCI, Carcinogenesis Program, Bethesda, MD; DHEW Pub # (NIH) 77-814, 1977.

##### Experimental design:

Lindane was administered in the diet of 50 Osborne-Mendel rats/sex/dose for a total of 80 weeks. Males received 320 or 640 ppm for 38 weeks and 160 or 320 ppm for the remaining 42 weeks. Females received 320 or 640 ppm for 2 weeks and 160 or 320 ppm for 49 weeks; then for the remaining 29 weeks, the dose was lowered to 80 or 160 ppm. After the initial 80 week treatment period, all animals were observed for an additional 29-30 weeks. Matched controls consisted of 10 rats/sex. For statistical analysis 45 untreated rats/sex were pooled from four other bioassays of other test chemicals.

##### Discussion of tumor data

As shown in Table 7, there were three incidences of spleen hemangioma in the high-dose male group only and none in the females. There were also increases in neoplastic lesions of the liver; however, these were within the historical control values in this tumor for this rat strain (0-12%, Goodman *et al.* 2000, personal communication). Other organs affected with primary tumors include: thyroid, pituitary, and mammary glands with only a few incidences and no clear dose-response correlation.

### Non-neoplastic Lesions

Microscopic changes were seen in the liver of both males and females, including cirrhosis, degeneration and necrosis in a dose dependent manner. Cysts, hyperplasia and atrophy were seen in the endocrine and reproductive organs of these animals.

**Table 7. Tumor data for Osborne-Mendel rats fed lindane for 80 weeks**

Dose	control males	low	high	control females	low	high
spleen hemangioma	0/8	0/44	3/44	-----	-----	-----
thyroid, adenoma carcinoma	1/6	5/37	0/37	0/8	1/44	1/42
	0/6	1/37	4/37	0/8	1/44	0/42
liver, neoplastic nodule	0/10	3/45	2/45	0/10	4/48	2/45
pituitary, adenoma carcinoma	0/10	0/32	2/35	0/7	0/45	2/41
	0/10	1/32	0/35	0/7	1/45	0/41
mammary, adenoma carcinoma	0/10	0/48	2/49	0/10	3/50	1/50
	0/10	1/48	0/49	1/10	1/50	0/50

### Adequacy of Dosing for Assessment of Carcinogenicity

Mean body weight did not show consistent changes from the administration of lindane. The CARC concluded that the use of pooled controls compromised the usefulness of the study and the available data were inadequate to make an assessment of the carcinogenic potential of lindane.

## IV. TOXICOLOGY

### **1. Metabolism**

Lindane is distributed to all organs at measurable concentrations within a few hours after oral administration. The highest concentrations are found in adipose tissue. The metabolism of lindane is initiated through one of the following pathways: dehydrogenation leading to HCH, dehydrochlorination leading to formation of PCCH, dechlorination leading to formation of -tetrachlorohexene, or hydroxylation leading to formation of hexachlorocyclohexanol. Further metabolism leads to a large number of metabolites. Volatilization appears to be an important route of its dissipation under the high-temperature conditions of tropical regions. Lindane is converted by enzymatic reactions, mainly in the liver. In mammals, including humans, lindane is excreted very rapidly in urine and feces after metabolic degradation; only small amounts are eliminated unchanged. The half-life of

lindane administered to rats is 2-4 days depending on the frequency of exposures, single or repeated.

Other metabolites are also known to be associated with lindane exposure; these include 2,4,6-trichlorophenol and 2,4,5-trichlorophenol. Exposure to lindane in a residential setting is expected to be negligible except for use as a lice or scabies treatment. These uses are regulated by FDA and have not been evaluated in this document.

## **2. Mutagenicity**

As part of the Registration Standard prepared in 1985, the available literature and submitted mutagenicity studies were evaluated (HED Document No.004704). Based on this evaluation, it was concluded that the weight-of-the-evidence with conventional genotoxicity testing indicated that lindane did not interact with DNA or interfere with genetic mechanisms. This position was reiterated in 1993 (memo by G. Ghali, 1993). Reviews prepared by IARC (1979/1987), IPCS (1990) and ATSDR (1999) indicate that lindane and its associated isomers have mixed genotoxic potential. In addition, numerous mutagenicity studies have been evaluated by the European Commission (EU) in their draft monograph on lindane (2001). Representative studies were selected from the EU evaluation of lindane since they were performed according to OECD or EPA guidelines. These include:

1. A bacterial mutagenicity assay using *Salmonella typhimurium* with and without metabolic activation which was negative up to cytotoxic doses (5000 ug/plate +S9) or insoluble doses (greater than or equal to 500 ug/plate -S9; greater than or equal to 1500 ug/plate +S9 ; Oesch, 1980).
2. An aerobic mammalian cell (V79) gene mutation assay was negative up to cytotoxic doses (greater than or equal to 50 ug/mL -S9; greater than or equal to 250 ug/mL+S9); the compound precipitated at greater than or equal to 250 ug/mL (Glatt, 1984). EPA only received an anaerobic assay which had an acceptable aerobic portion (Glatt, 1985).
3. Mammalian cell cytogenetic assay was negative in CHO cells up to cytotoxic doses (greater than or equal to 33.2 ug/mL -S9; greater than 33.2 ug/mL+S9). (Murli, 1990).
4. UDS in primary rat hepatocytes was also found to be negative up to cytotoxic doses (15 ug/mL) (Cifone, 1990).

There are no acceptable *in vivo* studies but they are not necessary to satisfy pre-1991 FIFRA guideline requirements. Newer published data shows that lindane induces oxidative stress in the liver of treated rats (Carrion *et al.*, 2001; Cornnejo *et al.*, 2001; Videla *et al.*, 2000). In agreement with these findings, the 1999 ATSDR review states that oxidative stress may be a possible mechanism of liver toxicity.

The CARC has an additional concern related to possible genetic effects on germinal cells.

Although an old submitted dominant lethal assay (MRID 00062657) was negative, it was considered unacceptable and not upgradable because of technical deficiencies. Recent information found in the open literature indicates that topical application of lindane led to rapid absorption and accumulation in rat testes (Suwalsky *et al.*, 2000). The investigators reported widespread damage to a "great number" of Leydig cells after the application of 1% lindane once daily for 4 consecutive days. These findings are consistent with the work of Walsh and Stocco (2000) showing inhibition of steroidogenesis by reductions in steroidogenic acute regulatory (StAR) protein expression in mouse Leydig cells *in vitro*. Since there is some evidence that lindane reaches and damages germ cells, the Committee recommends that the dominant lethal assay be repeated to determine if there is a genetic component to the reproductive (germ cell) effects reported above for lindane.

### 3. Structure-Activity Relationship

Technical-grade hexachlorohexane (HCH) which consists of alpha, beta, gamma, delta and epsilon isomers of HCH is a carcinogen. In rodents studies, the pure alpha isomer of HCH has been found to induce liver tumors; this is also true for the pure beta isomer of HCH.. These studies are found in the NTP, IARC and IPCS reports on hexachlorohexanes. Lindane shares its structure with at least four other isomers. They differ only with respect to the position of the chlorine atoms in the alpha or beta positions, above or below the plane of the hexane ring structure.

Structure of HCH (Lindane)::

-isomer: 1 2 3 hexachlorocyclohexane

Other isomers:

-isomer: 1 2 3 hexachlorocyclohexane

-isomer: 1 2 3 hexachlorocyclohexane

-isomer: 1 2 3 hexachlorocyclohexane

-isomer: 1 2 3 hexachlorocyclohexane

Isomers of hexachlorocyclohexane (HCH), other than lindane, have been classified as follows, according to IRIS: The technical HCH and the alpha-isomer are classified as B2, probable human carcinogens. The beta-isomer is classified as C, possible human carcinogen. The delta and epsilon isomers are classified as D, not classifiable as to human carcinogenicity.

Appendix A contains a summary of various studies which examine the carcinogenicity of the gamma isomer (Lindane) alone or in comparison with the other isomers.

### 4. Subchronic, and Chronic Toxicity

Subchronic Toxicity

Mice

A range-finding subchronic toxicity study (MRID 45424301) was conducted to

determine the doses to be used in a two-year carcinogenicity study. In this study, Lindane (99.78% a.i.) was administered to 10 CD-1 mice/sex/dose in the diet at dose levels of 0, 40, 80, 160, 320 ppm (0, 5.7, 12.2, 22.8 and 46.2 and 0, 8.9, 16.0, 32.9, and 62.6 mg/kg/day in males and females, respectively).

No treatment-related clinical signs were observed. Five females died or were killed during the treatment period. Four were in the highest dose (320 ppm) group and one from the control group was a humane kill. These animals presented with no macroscopic changes. Histopathology revealed hepatocyte hypertrophy and karyomegaly in the liver and Clara cell hypertrophy and congestion in the lungs. These findings were also seen in treated animals in the 160 and 320 ppm dose groups that were sacrificed at study termination; therefore, these deaths were considered to be treatment-related. Body weight was reduced by 6% in males and was unaffected in females in the highest dose tested (320 ppm). Body weight gain was reduced by 27% in males and 9% in females.

In a subchronic inhalation toxicity study, Lindane (99.6% a.i., Batch no. DA433) was administered by inhalation to groups of 45 male and 45 female CD-1 mice at nominal concentrations of 0, 0.3, 1.0, 5.0 to 10 mg/m<sup>3</sup> (0, 0.1, 0.4, 2.0 or 4.0 mg/kg/6 hrs), for 14 weeks. Exposures were 6 hours/day, 5 day/week as described in the pilot study. During the first five exposures, the high-dose group was exposed to a mean concentration of 9.72 mg/m<sup>3</sup> (4.0 mg/kg/6 hrs), but due to excessive deaths, the mean concentration was lowered to 4.94 mg/m<sup>3</sup> (2.0 mg/kg/6 hrs). No exposure-related effects were noted for body weight gain, food consumption, water consumption, or ophthalmoscopic, hematology, clinical chemistry, or urinalysis parameters. Bone marrow analysis did not show any time- or concentration-related changes. Brain, kidney, lung, spleen, thymus, and adrenal, and testes weights were similar between the treated and control animals. Liver weights of females exposed to 5 mg/m<sup>3</sup> were increased 14% (p = 0.05) at week 20.

#### Rats

In a subchronic oral neurotoxicity study (MRID 44781101), groups of 10 CrI:CD®BR rats/sex/group were administered lindane (Batch No. HLS96/1, Purity 99.78%) in the diet for 13 weeks at concentrations of 0 (control), 20, 100, or 500 ppm. Due to severe toxic reactions to treatment at 500 ppm, the dose was reduced to 400 ppm on day 11 of treatment thereafter. These doses resulted in average daily intake values of 0, 1.4, 7.1, and 28.1 mg/kg/day for males and 0, 1.6, 7.9, and 30.2 mg/kg/day in females for 0, 20, 100, and 500/400 ppm, respectively.

Significant treatment-related decreases (p<0.05 or p<0.01) in body weight were observed among males and females treated with 500/400 ppm of 14% and 23%, respectively. Decreases in body weight gains (70% and 180% , p<0.01), food consumption (35% and 50% , p<0.05 or p<0.01, respectively), and food conversion ratios were observed for males and females in the 500 ppm groups compared to the control group for the first week of the study. Male rats tended to recover from these effects after the dose was lowered. Females, however, did not

exhibit this same level of recovery as their food consumption remained slightly depressed throughout the remainder of the study.

Females in the 100 ppm group had significantly decreased body weight gains (40%,  $p < 0.05$ ) compared to the control group during the first week of the study and this effect continued, although not at a level of significance throughout the remainder of the study. Females in the 100 ppm group had significantly decreased food consumption (16%,  $p < 0.01$ ) for the first week of the study and this trend continued throughout the study. Liver weights were also found to be increased at 500/400 ppm for both sexes; no additional information was given.

### Chronic Toxicity

In the chronic toxicity/carcinogenicity study (MRID 41853701), lindane (99.75% a.i., Lot no. DA433) was administered in the diet to groups of 50 male and 50 female Wistar rats at concentrations of 0, 1, 10, 100, or 400 ppm for 2 years. Corresponding delivered doses were 0, 0.05, 0.47, 4.81, and 19.66 mg/kg/day, respectively, for males and 0, 0.06, 0.59, 6.00, and 24.34 mg/kg/day, respectively, for females. An additional 15 rats/sex/group were designated for interim sacrifices at 30 days and 26 weeks.

Body weights were slightly less than the controls for the high-dose males (-6%) and females (-8%) during weeks 1-5 of the study, but gradually increased to within 2% of the control level by week 26 for males and week 9-10 for females.

High-dose females had significantly decreased hemoglobin, decreased RBC counts, and decreased PCV. These red cell parameters were "marginally lower" for high-dose males (non statistically significant). Platelet counts increased in mid- and high-dose males and females. White cell counts significantly increased in mid-dose and in high-dose females due to increases in neutrophils.

The liver appears to be the major target organ. Kidney lesions in male rats indicative of alpha 2 globulin accumulation were observed in animals treated with 10 ppm, but are not considered relevant to human health risk assessment. Absolute kidney weights were significantly increased in high-dose males. Absolute and relative kidney weights increased in mid-dose males and high-dose males and females. The incidence of periportal hepatocytic hypertrophy was significantly increased in males at 100 and 400 ppm and in females at 400 ppm. This lesion was not seen in control animals of either sex. No treatment-related histopathological lesions were observed in the spleen, adrenals, brain, or thymus. Bone marrow data presentation was inadequate for assessment.

## 5. Mode of Action Studies

No mode of action studies have been submitted for lindane. There have been, however, several published studies which attempt to elucidate the initiator-promoter



activity of lindane. As discussed earlier lindane does not appear to have a clear mutagenic potential.

Lindane may act as a promoter as evidenced by studies with Agouti, Pseudoagouti and Black mice. Only Agouti and Pseudoagouti mice, which have a transformed genotype linked to tumorigenicity, were found to have an increased incidence of liver and lung tumors. The Black mice had no tumors in the 24 month period of the study. The Pseudoagouti and Black mice had a low rate of spontaneous tumor incidence in the liver and lung, but of these two only the Pseudoagouti responded to lindane. Therefore, lindane appears to augment the propensity of these genetically altered mice to develop tumors. It has been suggested that lindane has a similar mode of action to phenobarbital, which also increases the incidence of benign tumors in these mice. As discussed earlier on page 13, oxidative stress may be a possible mechanism of liver toxicity.

Suggestions have been made that the metabolites of lindane may contribute to its carcinogenic potential. One major urinary metabolite, 2,4,6-TCP, is considered to be a carcinogen. However studies indicate that TCP may have only a minimal effect on the overall carcinogenic potential of lindane.

## V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

### 1. Carcinogenicity

**The CARC concluded that lindane is carcinogenic only to female mice and is not carcinogenic to male mice and male and female rats.**

- CD-1 female mice had significant increasing trends and significant differences in pair-wise comparisons of the 160 ppm (26.8 mg/kg/day) dose group with the controls, for lung alveolar-bronchiolar adenomas (23% vs 6% in controls) and combined adenomas/carcinomas (25% vs 8% in controls), all at  $p < 0.05$ ; the incidence of lung adenomas (23%) was slightly outside the historical control range (6%- 19%). The increased incidence of carcinomas was not dose-dependent and tumor response was variable. Lindane was not carcinogenic to male mice. No non-neoplastic liver changes were seen in females. The majority of the CARC considered the dosing to have been adequate and not excessive based on an increase in the incidence of centrilobular hepatocyte hypertrophy as well as eosinophilic foci of hepatocellular alteration in high-dose males compared to the control group; similar liver findings were also reported in a range-finding subchronic toxicity study in which early deaths of four females were reported in the 320 ppm dose group indicating that this dose was excessive. However, based on the results of the chronic study a few members felt that the animals could have tolerated a higher dose.

- At 160 ppm, both the treated female Agouti and Pseudoagouti mice had an increased occurrence of benign lung tumors (19% vs 4% in controls and 14% vs 6% in controls, respectively). In addition, both the treated female Agouti and Pseudoagouti mice had increases in liver adenomas (35% vs 9% in controls and 17% vs 13% in controls, respectively) and slight increases in liver carcinomas (5% vs 2% in controls and 12% vs 5% in controls) at 24 months. No statistical analyses of tumor data were provided. There was no increase in incidence or decrease in latency period of liver tumors in Black and Pseudoagouti strains of mice. There was evidence of increased liver weights and increased incidence of Clara cell hyperplasia in Agouti and Black strains of mice. Increases in Clara cell hyperplasia were noted in the lung at all sacrifice intervals for each strain and the incidence of lung tumors was increased in later months for the female Agouti and Pseudoagouti mice. The percentage of mice with Clara cell hyperplasia in the treated and control groups was 72%-92% and 6%-31%, respectively, for the Agouti; 50%-79% and 6%-17%, respectively, for the Pseudoagouti; and 56%-90% and 0%-14%, respectively, for the female Black mice. However, the study was conducted on few animals, only a single dose was tested, no statistical analyses of tumor data were presented; and the results of the study were not adequately reported. The Committee concluded that although the liver effects appear to suggest that a dose of 160 ppm was adequate, additional dose groups could have provided confirmatory information.
- The incidence of hepatocellular carcinoma in low-dose B6C3F<sub>1</sub> males (19/49) was significant ( $p=0.001$ ) when compared with that in pooled controls (5/49). The incidence of hepatocellular carcinoma in high-dose male mice (9/46) was not significantly different than the matched (2/10) or pooled controls. The CARC could not assess the carcinogenicity of lindane in B6C3F<sub>1</sub> male and female mice because the data reporting was inadequate, there were no indications of toxicity at the high dose and the test material could not be validated. Moreover, the use of only 10 mice per sex for the control group compromised the usefulness of the study.

**The Committee concluded that the increased incidence of benign lung tumors in female CD-1 mice was treatment-related** because the treatment-related statistically significant increase in lung adenomas in female CD-1 mice was correlated with increases in lung tumors in two genetically susceptible strains of mice (Agouti and Pseudoagouti). Although there is some evidence of liver tumor induction in these genetically susceptible strains of mice, there was no evidence of liver tumors in CD-1 mice. Nevertheless, the evidence of hepatotoxicity (increased incidences of liver hypertrophy and liver foci in both sexes) and promoting activity suggests the liver as a major target organ of toxicity.

- The treated male Wistar rats developed adrenal pheochromocytomas. The percentages of animals with adrenal tumors in the 0, 1, 10, 100, and 400 ppm groups were 14%, 16%, 16%, 6%, and 24% for benign tumors, respectively, and 0%, 0%, 6%, 8%, and 2% for malignant tumors, respectively. Statistical

significance was not reached by relevant tests and no dose-response was evident. When compared to historical controls, the incidence of adrenal pheochromocytomas in the current study slightly exceeded that of the historical control at the HDT (400 ppm). The range of adrenal pheochromocytomas observed in the historical control data was 4/50 to 11/50 (8% - 22%) for male rats examined in four studies conducted in 1990. Of the 18 studies in the historical control data, 6 were performed in 1990; the other 12 were performed between 1986 and 1988. **The Committee concluded that the adrenal tumors in male rats were not treatment-related.** The doses tested were considered to be adequate and not excessive in both sexes based on decreased survival, decreased body weight gains and decreased food consumption; the increased spleen and liver weights correlated with increased occurrence of periacinar hepatocyte hypertrophy in both sexes at the high-dose.

- There were three spleen hemangiomas in 44 high-dose male Osborne-Mendel rats only (0/8 in controls) and none in the females. There were also non-dose related increases in neoplastic lesions of the liver (3/45 and 2/45 in males and 4/48 and 2/45 in females in the low and high dose groups compared to 0/10/sex in control groups) which were within the historical control values (0%-12%). Other organs with primary tumors include: thyroid, pituitary, and mammary glands with only a few incidences but there was no clear dose-response. Dosing at the highest level was considered to be adequate based on microscopic changes seen in the liver of both males and females, including cirrhosis, degeneration, necrosis in a dose dependent manner. Cysts, hyperplasia and atrophy were seen in the endocrine and reproductive organs of these animals. Survival of the animals was adequate for meaningful statistical analyses of the incidence of tumors.

**The CARC concluded that lindane was not carcinogenic to male and female Wistar rats and that the results of the study in Osborne Mendel rats were difficult to interpret and were not useful in determining the carcinogenic potential of lindane in that strain of rat.**

## 2. Mutagenicity

- Lindane has been tested in a battery of pre-1991 mutagenicity assays which satisfies the guideline requirements. The review of both the guideline and literature studies suggests that lindane does not interact with DNA or interfere with genetic mechanisms. However, since there is some evidence that lindane reaches and damages germ cells, the Committee recommended that the dominant lethal assay be repeated to determine if there is a genetic component to the reproductive (germ cell) effects reported for lindane.

## 3. Structure Activity Relationship

- Isomers of hexachlorocyclohexane (HCH), other than lindane, have been

classified for carcinogenic potential. The technical HCH and the alpha-isomer are classified as B2, probable human carcinogens. The beta-isomer is classified as C, possible human carcinogen. The delta and epsilon isomers are classified as D, not classifiable as to human carcinogenicity.

#### 4. Mode of Action

- The tumor-initiating activity reported in the literature has been discounted due to the lack of morphologic alterations in liver foci after treatment with lindane. Lindane appears to augment the propensity of genetically altered mice to develop tumors. However, no definitive studies have established the mode of action for liver tumor induction by lindane. There is a suggestion that oxidative stress may play a role in the liver toxicity of lindane.

### VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the Agency's *Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the Committee classified lindane into category: **"Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential"** based on the occurrence of benign lung tumors in one sex of one species (i.e., female CD-1 mice).

### VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended that quantification of human cancer risk is not required.

**VIII BIBLIOGRAPHY**

<u>MRID No.</u>	<u>CITATIONS</u>
00160863	Koenig, GR, Rexroat, MA and Probst, GS (1985) The effect of benefin (EL-110, compound 54521) on the induction of reverse mutations of <i>Salmonella typhimurium</i> using the Ames test. Study Laboratory: Toxicology Div Lilly Research Laboratories, Laboratory# 85624UB2598 and 850708UB 2598, August 13, 1985.
00160865	Koenig, GR, Hill, LE and Probst, GS (1985) The effect of benefin (EL-110, Compound 54521) on the induction of DNA synthesis in primary cultures of adult rat hepatocytes. Study Laboratory: Toxicology Div Lilly Research Laboratories, Laboratory# 85716UDS2598 and 850723UDS2598, October 29, 1985.
00160866	Koenig, G.R., Oberly, T.J., Bewsey, B.J., (1985) The Effect of Benefin (EL-110, Compound 54521) on the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells. Toxicology Division, Lilly Research Laboratories, Greenfield, IN. Laboratory Study Numbers 850612MLA2598 and 850724MLA2598, October 1985. Unpublished.
40693201	Berard, D.F. (1988) Characterization and Identification of Radioactivity in Urine and Feces of Rats Dosed with <sup>14</sup> C Benefin. Lilly Research Laboratories, Greenfield, IN. Laboratory Project Id. ABC-0389, April 4, 1988. Unpublished.
40693202	Koenig, G.R., Pohland, R.C. (1988) Excretion of Radiocarbon in the Expired Air of Fischer 344 Rats Given a Single Oral Dose of <sup>14</sup> C-Benefin (EL-110, Compound 54521). Lilly Research Laboratories, Greenfield, IN. Laboratory Project Id. R06087, May 25, 1988. Unpublished.
40693203	Koenig, G.R., Byrd, T.K., Pohland, R.C. (1988) Radiocarbon Disposition in Fischer 344 Rats Given Single Oral Doses of <sup>14</sup> C-Benefin (EL-110, Compound 54521): Pharmacokinetics, Excretion, and Residual Tissue Levels. Lilly Research Laboratories, Greenfield, IN. Laboratory Project Id. R16687, May 25, 1988. Unpublished.
40693204	Koenig, G.R., Byrd, T.K., Pohland, R.C. (1988) Biliary Excretion of Radioactivity by Fischer 344 Rats Given Single Oral Doses of <sup>14</sup> C-Benefin (EL-110, Compound 54521). Lilly Research Laboratories, Greenfield, IN. Laboratory Project Id. R09887 and R23887, May 25, 1988. Unpublished.
40693205	Koenig, G.R., Byrd, T.K., Pohland, R.C. (1988) Tissue Distribution of Radioactivity in Fischer 344 Rats Given Single Oral Doses of <sup>14</sup> C-Benefin (EL-110, Compound 54521). Lilly Research Laboratories, Greenfield, IN.

Laboratory Project Id. R09887, May 25, 1988. Unpublished.

45291402. Chase, K. (2000) Lindane, carcinogenicity study by dietary administration to CD-1 mice for 78 weeks, final report (vols. 1-4). Huntingdon Life Sciences Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England, Report no. 00 3512, Huntingdon Life Sciences Project identity no. CIL/021, December 20, 2000.
42891201. Aymes, S.J. 1993. Lindane: Combined carcinogenicity and toxicity study by dietary administration to Wistar rats for 104 weeks. Addendum to final report (Adrenal histopathology - additional investigations). Life Sciences Research, England. Study No. 90/CIL002/0839. June 2, 1993
- 45470601 Huntingdon Life Sciences Ltd. Additional histopathology investigations of female mouse lung tissues conducted by Huntingdon Life Sciences Ltd., Cambridgeshire, England, for C.I.E.L. (Centre International Etudes du Lindane), Brussels, Belgium, and completed July 31, 2001 (Project Identity No. CIL/027.
00162724. Huntington Research Center (Lindane toxicity study in beagle dogs) report #3720/70/532, 1970
- Boehringer C. H. Sohn Ingelheim am Rhein.; (Testing of the substance Lindane for carcinogenic effects in mice using oral administration- duration 80 weeks) translated from German (1975).
- Brunsman, L.L. ; Lindane: Qualitative Risk Assessment Based On Crl:CD-1 (ICR) BR Mouse Dietary Study. Memorandum from Lori Brunsman, Science Information Management Branch, to Suhair Shallal, Reregistration Branch 4, Health Effects Division, Office of Pesticide Programs, Environmental Protection Agency, dated May 1, 2001. HED Doc.# 014556.
- Brunsman, L.L. ; ADDENDUM To Lindane: Qualitative Risk Assessment Memo of 5/1/2001 Based On Additional Histopathology Investigations of Female Lung Tissues of Crl:CD-1(ICR) BR Mouse Dietary Study. Memorandum from Lori Brunsman, Science Information Management Branch, to Suhair Shallal, Reregistration Branch 4, Health Effects Division, Office of Pesticide Programs, Environmental Protection Agency, dated August 16, 2001. HED Doc.# 014652.
- Fitzhugh, O.G.; Nelson, A.A.; and Frawley, J.P.; The chronic toxicities of technical benzene hexachloride and its alpha, beta and gamma isomers J. Pharmacol. Expt. Therapeutics. 100: 59 (1950)..
- Goto, M.; Hattori, M.; Miyagawa, T.; and Enomoto, M.; Contributions to ecological chemistry II. Hepatoma development in mice after administration of HCH isomers in high dosage Chemosphere 1(6): 279-282 (1972).

- Hanada, M.; Yutani, C.; and Miya, T.; Induction of hepatoma in mice with benzene hydrochloride GANN 64:511-513 (1973).
- Ito, N.; Nagasaki, H.; Arai, M.; Sugihara, S.; and Makiura, S.; Pathologic and ultrastructural studies in the hepatocarcinogenicity of benzene hexachloride in mice J. NCI 51:817-826 (1973).
- Ito, N.; Nagasaki, H.; et al.; Brief communication: development of hepatocellular carcinomas in rats treated with benzene hexachloride J. NCI 54:801-805 (1975).
- Lindane, Environmental Health Criteria 124, IPCS, WHO, Geneva, Switzerland (1991).
- Nagasaki, H.; Tonrii, S.; et al. ; Carcinogenicity of Benzene Hexachloride (BHC) Proc. of 2<sup>nd</sup> Intern Symp of Princess Tokamatsu Cancer Center Res. Fund in Topics in Chem. Carcino. 1972
- NCI.; NTP Combined Chronic Toxicity/Carcinogenicity Study with Lindane in Mice. National Cancer Institute, Carcinogenesis Program, Bethesda, MD; DHEW Pub # (NIH) 77-814 (1977).
- NCI; NTP Combined Chronic Toxicity/Carcinogenicity Study with Lindane in Rats. National Cancer Institute, Carcinogenesis Program, DHEW Pub # (NIH) 77-814 (1977).
- Ortega, P.; Hayes, W.J.; and Durham, W.F.; Pathologic changes in the liver of rats after feeding low levels of various insecticides A.M.A. Archives of Pathology, 64:614, (1957).
- Thorpe and Walker; The toxicology of dieldrin (HEOD). II. Comparative long-term oral toxicity studies in mice with dieldrin, DDT, phenobarbitone, beta-BHC and gamma-BHC Fd. Cosmet. Toxicol, 11:433-442 (1973).
- Wolff, G.L., Roberts, D.W., Morrissey, R.L., Greenman, D.L., Allen, R.R., Campbell, W.L., Bergman, H., Nesnow, S., and Firth, C.H. 1987. Tumorigenic responses to lindane in mice: potentiation by a dominant mutation. Carcinogenesis 8:1889-1897 (1987). National Center for Toxicological Research, Jefferson, AK.

## APPENDIX A

### Carcinogenicity Studies comparing the toxicity of gamma-HCH with other isomers

Study	# of animals	doses	results for lindane (gamma-HCH)	results for other isomers
Carcinogenicity- rat (The chronic toxicities of technical benzene hexachloride and its alpha, beta and gamma isomers) published:1950	10 / 10 Wistar rat	0, 5, 10, 50, 100, 400, 800 or 1600 ppm of , or HCH.	At 100 ppm of HCH , liver wt. incr no frank liver tumors induced by HCH	
Carcinogenicity-Rats (Pathologic changes in the liver of rats after feeding low levels of various insecticides) published: 1957	6 / 6 ~	0, 50, 100 ppm for 8 months.	One 50 ppm , and one each 100 ppm and developed centrilobular hypertrophy, peripheral migration of basophilic cytoplasmic granulations and cytoplasmic inclusion bodies.	
Carcinogenicity-rats (Brief communication: development of hepatocellular carcinomas in rats treated with benzene hexachloride) published: 1975.	9 groups of W rats (Japanese strain)	0, 500, 1000 or 1500 ppm of or BHC	<b>BHC</b> : cell hypertrophy	<b>BHC</b> : cell hypertrophy, nodular hyperplasia (27/41 dosed w/ 1000 ppm for 48-72 wks), hepatocellular carcinoma (4/29 dosed w/ 1000 ppm for 72 wks) :cell hypertrophy



Carcinogenicity-rats (Bioassay of lindane for possible carcinogenicity) NCI (NCI-RG-TR-14) 1977	10 / 10 Osborne Mendel rats 50 ----  50 ----	0 ppm;  320 or 640 ppm for 38 wks, 160 or 320 ppm for 42 wks, then 0 ppm for 30 wks  320 or 640 for 2 wks, 160 or 320 ppm for 49 wks, 80 or 160 ppm for 29 wks, then 0 ppm for 30 wks.	Incidence of liver neoplasia is within historical control levels	
Carcinogenicity-mice (Bioassay of lindane for possible carcinogenicity) NCI (NCI-RG-TR-14) 1977	10 / 10 BGC3F1 hybrid mice  50 / 50	0 ppm  80 or 160 ppm for 80 wks then control diet for 10 wks	hepatocellular carcinoma: 0 ppm (20%), 80 ppm (39%), 160 ppm (20%)	
Carcinogenicity-mouse (The toxicology of dieldrin (HEOD). II. Comparative long-term oral toxicity studies in mice with dieldrin, DDT, phenobarbitone, beta-BHC and gamma-BHC) Thorpe and Walker (1973).	CF1 mice 45 / 45  30 / 30	0 ppm (24/23%)  400 ppm (gamma-BHC) 10 ppm (dieldrin) 100 ppm (DDT) 500 ppm (phenobarbitone) 200 ppm (beta-BHC)	/ % of liver tumors (gamma-BHC) 93/69%	/ % of liver tumors (dieldrin)100/ 87% (DDT) 77/87 % (phenobarbitone) 80/75% (beta-BHC) 73/43 %
Carcinogenicity-mouse (Testing of the substance Lindane for carcinogenic effects in mice using oral administration-duration 80 weeks) translated from German 1975	SPF mice (Chbb-NMRI) 100 / 100  50 / 50	0 ppm  12.5, 25 or 50 ppm for 80 wks	incidences ( / ) liver cell adenomas: 4/1, 1/1, 0/0, 2/0 lung tumor: 13/8, 10/1, 5/3, 6/4 lymphosarcoma: 5/12, 0/7, 1/3, 2/5, respectively	

Carcinogenicity-mouse (Pathologic and ultrastructural studies in the hepatocarcinogenicity of benzene hexachloride in mice) published (1973)	19 groups (20-40/group)	or BHC at 100, 250, or 500 ppm and combos of 250 ppm ea. of with , or BHC.		Only mice that were dosed w/ BHC in combo. or alone developed nodular hyperplasia and hepatocellular carcinoma
Carcinogenicity-mouse (Contributions to ecological chemistry II. Hepatoma development in mice after administration of HCH isomers in high dosage. Published: (1972).	8 groups of 20 ICR-JCL mice	600 ppm of technical HCH (I), HCH (II) HCH (III), HCH (IV), a mix of HCH (V), or 300 ppm of HCH (IX)	5/10 in gp IX developed liver tumors and incr. Liver wgt.	all of group I and II developed hepatomas 8/10 in gp V developed liver tumors and incr. Liver wgt.
Carcinogenicity-mouse (Carcinogenicity of Benzene Hexachloride (BHC)) published: (1972)	4 groups of dd mice	0, 6.6, 66, 660 ppm of technical BHC (67%), (11%), (15%) or (6%) BHC and other isomers < 1% for 24 wks		at all doses: liver wgt, cellular hyperplasia, nodular hyperplasia, all 660 ppm mice developed hepatoma
Carcinogenicity-mouse (Induction of hepatoma in mice with benzene hydrochloride) published: (1973).	4 groups of dd mice	0, 100, 300 or 600 ppm with crude BHC, or pure or isomer for 36-38 wks	No control and 100 or 300 ppm isomer mice developed hepatomas	many mice in crude BHC, or pure isomer developed hepatoma,
Chronic feeding-Dogs (Lindane toxicity study in beagle dogs) 1970	4 group of 4 beagles/sex	0, 25, 50, or 100 ppm for 104 wks	NOAEL: 50 ppm LOAEL: 100 ppm liver changes, slight inc liver wt.	